

CORRECTED
VERSION*

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

| | | | |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------|
| (51) International Patent Classification ⁷ : C12N 15/12, C07K 14/47, G01N 33/50, A01K 67/027, A61K 38/17 | | A3 | (11) International Publication Number: WO 00/18917 (43) International Publication Date: 6 April 2000 (06.04.00) |
| (21) International Application Number: PCT/US99/22302 (22) International Filing Date: 27 September 1999 (27.09.99) (30) Priority Data: 60/101,862 25 September 1998 (25.09.98) US 09/405,035 24 September 1999 (24.09.99) US (71) Applicant: AMGEN INC. [US/US]; One Amgen Center Drive, Thousand Oaks, CA 91320-1799 (US). (72) Inventors: BIERE, Anja, Leona; 1272 Wilder Street, Thousand Oaks, CA 91362 (US). CITRON, Martin; 1272 Wilder Street, Thousand Oaks, CA 91362 (US). (74) Agents: ODRE, Steven, M. et al.; Amgen Inc., One Amgen Center Drive, Thousand Oaks, CA 91320-1799 (US). | | (81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published With international search report. (88) Date of publication of the international search report: 2 November 2000 (02.11.00) | |
| (54) Title: α -SYNUCLEIN SUPER-MUTANTS ACCELERATE α -SYNUCLEIN AGGREGATION (57) Abstract <p>Parkinson's disease (PD) is a neurodegenerative disorder which is pathologically characterized by the presence of intracytoplasmic Lewy bodies, the major component of which are filaments consisting of α-synuclein. The present invention provides α-synuclein mutations which accelerate α-synuclein aggregation and can thus be utilized for transgenic animal production and generation of the first progressive PD model. Also provided is an <i>in vitro</i> aggregation assay which can be utilized to identify α-synuclein nucleation inhibitors for the treatment of PD.</p> | | | |

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

| | | | | | | | |
|----|--------------------------|----|------------------------------------------|----|----------------------------------------------|----|--------------------------|
| AL | Albania | ES | Spain | LS | Lesotho | SI | Slovenia |
| AM | Armenia | FI | Finland | LT | Lithuania | SK | Slovakia |
| AT | Austria | FR | France | LU | Luxembourg | SN | Senegal |
| AU | Australia | GA | Gabon | LV | Latvia | SZ | Swaziland |
| AZ | Azerbaijan | GB | United Kingdom | MC | Monaco | TD | Chad |
| BA | Bosnia and Herzegovina | GE | Georgia | MD | Republic of Moldova | TG | Togo |
| BB | Barbados | GH | Ghana | MG | Madagascar | TJ | Tajikistan |
| BE | Belgium | GN | Guinea | MK | The former Yugoslav Republic of Macedonia | TM | Turkmenistan |
| BF | Burkina Faso | GR | Greece | ML | Mali | TR | Turkey |
| BG | Bulgaria | HU | Hungary | MN | Mongolia | TT | Trinidad and Tobago |
| BJ | Benin | IE | Ireland | MR | Mauritania | UA | Ukraine |
| BR | Brazil | IL | Israel | MW | Malawi | UG | Uganda |
| BY | Belarus | IS | Iceland | MX | Mexico | US | United States of America |
| CA | Canada | IT | Italy | NE | Niger | UZ | Uzbekistan |
| CF | Central African Republic | JP | Japan | NL | Netherlands | VN | Viet Nam |
| CG | Congo | KE | Kenya | NO | Norway | YU | Yugoslavia |
| CH | Switzerland | KG | Kyrgyzstan | NZ | New Zealand | ZW | Zimbabwe |
| CI | Côte d'Ivoire | KP | Democratic People's Republic of Korea | PL | Poland | | |
| CM | Cameroon | KR | Republic of Korea | PT | Portugal | | |
| CN | China | KZ | Kazakhstan | RO | Romania | | |
| CU | Cuba | LC | Saint Lucia | RU | Russian Federation | | |
| CZ | Czech Republic | LI | Liechtenstein | SD | Sudan | | |
| DE | Germany | LK | Sri Lanka | SE | Sweden | | |
| DK | Denmark | LR | Liberia | SG | Singapore | | |
| EE | Estonia | | | | | | |

INTERNATIONAL SEARCH REPORT

International Application No
PC US 99/22302

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/12 C07K14/47 G01N33/50 A01K67/027 A61K38/17

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C07K A01K A61K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| A | WO 95 06407 A (SAITOH TSUNAO ;MASLIAH ELIEZER (US); UNIV CALIFORNIA (US)) 9 March 1995 (1995-03-09) abstract examples 1-16 seq. ID 2 claims 1-92 | 1,2,4-6 |
| A | --- POLYMEROPOULOS M H ET AL: "Mutation in the alpha-synuclein gene identified in families with Parkinson's disease" SCIENCE,US,AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE,, vol. 276, 27 June 1997 (1997-06-27), pages 2045-2047, XP002083895 ISSN: 0036-8075 --- -/-- | 1,2,4-6 |

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"Z" document member of the same patent family

Date of the actual completion of the international search

9 February 2000

Date of mailing of the international search report

24.07.00

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Galli, I

INTERNATIONAL SEARCH REPORT

International Application No

PC., JS 99/22302

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| A | PAIK S.R. ET AL.: "Self-oligomerization of NACP, the precursor protein of the non-amyloid beta/A4 protein (A beta) component of Alzheimer's disease amyloid, observed in the presence of C-terminal A beta fragment (residues 25-35)" FEBS LETTERS, vol. 421, no. 1, 2 January 1998 (1998-01-02), pages 73-76, XP002130118 the whole document --- | 1,2,4-6 |
| A | SPILLANTINI ET AL: "alpha synuclein in Lewy bodies" NATURE,GB,MACMILLAN JOURNALS LTD. LONDON, vol. 388, 28 August 1997 (1997-08-28), pages 839-840, XP002090996 ISSN: 0028-0836 cited in the application the whole document --- | 1,2,4-6 |
| A | GOEDERT M: "The awakening of alpha-synuclein" NATURE,GB,MACMILLAN JOURNALS LTD. LONDON, vol. 388, 17 July 1997 (1997-07-17), pages 232-233, XP002083894 ISSN: 0028-0836 ----- | 1,2,4-6 |

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 99/22302

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Claim 7 is directed to a method of treatment of the human/animal body: a search can be performed only based on the alleged effects of the compound/composition, if described.
2. ☒ Claims Nos.: 7
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Claims 1, 2, 4 completely, Claims 5 - 7 partially.

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box 1.2

Claims Nos.: 7

Claim 7 refers to agents affecting nucleation of alpha-synuclein without giving a true technical characterization. Moreover, no such compounds are defined in the application. In consequence, the scope of said claim is ambiguous and vague, and its subject-matter is not sufficiently disclosed and supported (Art. 5 and 6 PCT). No search can be performed for such purely speculative claims whose wording is, in fact, a mere recitation of the results to be achieved.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: (1,2,4) - completely; (5-7) - partially

An isolated artificial alpha-synuclein mutant polypeptide which demonstrates accelerated aggregation to naturally occurring alpha-synuclein mutant polypeptides.

Said mutant being represented by seq. IDs 2,3 and 4.

A transgenic non-human animal engineered to express said polypeptide in the brain.

A method in which said polypeptide is used to screen for alpha-synuclein nucleation inhibitors.

2. Claims: (3) - completely

An in vitro aggregation assay for evaluating alpha-synuclein mutants which demonstrate accelerated aggregation.

3. Claims: (5-7) - partially

A method in which alpha-synuclein or aggregation-competent fragments thereof are used to screen for alpha-synuclein nucleation inhibitors.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PC JS 99/22302

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|-------------------------------------------|---------------------|----------------------------|---------------------|
| WO 9506407 | A | 09-03-1995 | NONE |
| ----- | | | |